

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

Claims 1-22 (cancelled)

Claim 23 (new): A process for the preparation of an agglutination reagent for rapid and early detection of typhoid comprising:

- (a) preparing antibody specific to *Salmonella typhi*;
- (b) preparing latex particles suspension;
- (c) coating of the said latex particles with the said antibody;

wherein the said process of preparing antibody specific to *Salmonella typhi* comprises cloning Flagellin gene sequence specific to *Salmonella typhi*, expressing the said Flagellin gene sequence by recombinant DNA technology, followed by purifying recombinant protein by affinity chromatography, raising the hyper immune sera against purified recombinant protein in animals like rabbit, separating the antibody (immunoglobulin) fraction of hyper immune sera by precipitating in ammonium sulphate, suspending in 50 mM phosphate buffer of pH 7.2 and dialyzing;

wherein the said process of preparing latex particle suspension comprises:

- (i) mixing 1% carboxylated latex particles of size 0.88 to 0.90 μm and 40 mM 2-N Morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1 on a vortex mixer for about 60 seconds, centrifuging at 10,000 rpm for 10-12 minutes at about 4°C, followed by washing twice with 20 mM MES buffer of pH 5.5 at 10,000 rpm for 10-12 minutes at about 4°C, sonicating by a tip sonicator at about 5 watts for 60-120 seconds;
- (ii) adding drop wise a freshly prepared solution of 0.1 M 1-ethyl-3 (3-dimethyl-amino propyl) carbodimide hydrochloride (EDC) in 20 mM MES buffer of pH 5.5 to the said sonicated latex particles obtained from step (i) above in a ratio of

1:1 while vortexing the suspension slowly, rotating the suspension slowly end-over-end for about 3 hours at a temperature of 20-25°C, washing thrice with 20 mM MES buffer (pH 5.5) followed by sonicating the washed suspension of latex particles by a tip sonicator for 60-120 seconds at about 5 watts;
wherein the said process of coating of the said latex particles is done by adding 0.6-1.0 mg preferably 0.8 mg per ml of the said antibody (immunoglobulins) to the said latex particle suspension, rotating the suspension end-over-end for 18-20 hours at a temperature of about 20-25°C, stopping the coating reaction by 1M glycine (pH 11.0) taken in quantity of 0.06 ml per ml of solution of antibody coated latex particles followed by centrifugation at 10,000 rpm for 10-12 minutes at a temperature of about 4°C, washing thrice with washing buffer comprised of 50 mM glycine, pH 8.5; 0.03% triton X-100 and 0.05% sodium azide, suspending in storage buffer to a final concentration of 1%, sonicating for about 60 seconds at about 5 watts and storing at 4°C.

Claim 24 (new): An agglutination reagent for rapid and early detection of typhoid, comprising of 1% carboxylated latex particles coated with antibody specific to *Salmonella typhi*, suspended in storage buffer.

Claim 25 (new): The agglutination reagent as claimed in claim 24, wherein the size of the said latex particles is 0.88 to 0.90 µm.

Claim 26 (new): The agglutination reagent as claimed in claim 24, wherein the said storage buffer is comprised of 50 mM glycine pH 8.5, 1.0% bovine serum albumin, 0.03% triton X-100, 0.1% sodium azide and 0.01% thiomersal.

Claim 27 (new): The agglutination reagent for rapid and early detection of typhoid as claimed in claim 24, wherein the said antibody is the immunoglobulin fraction, of the hyper immune sera raised in rabbit against the recombinant protein expressed by cloning of Flagellin gene sequence specific to *Salmonella typhi* by recombinant DNA technology, suspended in 50 mM phosphate buffer.

Claim 28 (new): A kit for rapid and early detection of typhoid comprising 1% agglutination reagent as claimed in claim 24 suspended in storage buffer, glass slides, droppers, wooden sticks and positive and negative controls.